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(71) Applicant: IDEXX LABORATORIES, INC. [US/US]; One Idexx Drive, Westbrook, ME 04092 (US).

(72) Inventors: EHRENFELD, Elizabeth; 6 Shoreline Drive, Falmouth, ME 04105 (US). CARPENTER, Charles; 175 Holmes Road, Scarborough, ME 04074 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

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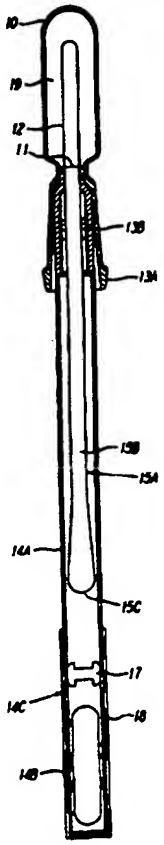
(54) Title: SELF-CONTAINED SIGNAL GENERATING SAMPLING DEVICE AND METHODS OF USE OF SAME

(57) Abstract

A self-contained signal generating device and methods for use of such a device are provided. The device and methods may detect the presence of biological or toxic materials and may utilize, among other compositions, a luciferin/luciferase composition, nutrient-indictor media or bioluminescent bacteria to provide a signal indicative of the presence of biological or toxic material.



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DESCRIPTION

SELF-CONTAINED SIGNAL GENERATING SAMPLING DEVICE AND METHODS OF USE OF SAME

Background of the Invention

This invention relates in general to a device and methods for generating a signal in response to the testing of a sample which may contain biological material or toxic material wherein the device generating the signal is self-contained.

The use of sampling devices in various fields is known. For example, the sampling for contamination of food processing facilities; the sampling of contamination of the environment by heavy metals such as lead or cadmium; the collection of specimens from a patient to test for microorganism infection.

Generally, the devices and methods previously used required various inconvenient steps. For example, batch preparation of a chemical which would provide a desired signal upon contact with a signal-generating sample, followed by pipetting of the batch preparation into multiple tubes. This preparation might take place in a food processing facility where contamination of the food being processed by foreign substances, such as a signal-generating chemical is a major concern. Often, for reasons of personnel turnover or otherwise, the personnel doing the sampling may lack significant experience preparing chemical formulations, thereby introducing the risk of error in preparation of the signal-generating composition. Other devices have required shipment back to laboratories for further processing and/or analysis of samples obtained. For example, food processing facilities often use bacterial plating and counting techniques which could take 24-48 hours to provide results. Environmental sampling for biological contamination often takes up to

five days.

Some devices have attempted to overcome these problems by designing a self-contained testing unit. However, when particular signal generating chemicals such as luciferase/luciferin are used their design is believed not to offer the same degree of stability or ease of use of the luciferase/luciferin as in the present invention. Further, due to the sensitive nature of the test for biological contamination, contamination of the testing device prior to use must be avoided. The present invention provides for the protection of a sampling means prior to use, whereas other devices leave the sampling means relatively accessible to pre-test contamination.

The demand for a self-contained, easily used, stable, and storable testing device, yielding immediate results is greater than ever. This demand has occurred in light of increased consumer and regulatory pressure, for example, in response to recent deaths and illness caused by contamination of beef at fast food restaurants and incidents involving contaminated cheese and milk products. The device of the present invention fulfills this need.

Summary of the Invention

This invention in one aspect features a device which allows for the rapid and convenient testing of surfaces, solutions or atmospheres for biological contamination by assaying for the presence of adenosine triphosphate ("ATP"). ATP is assayed for because it is a ubiquitous cellular component in both procaryotes and eucaryotes. For example, in one application, the device will allow for the testing of surfaces in, for example, food processing factories, supermarkets and fast food restaurants to determine if such surfaces contain biological material. In another embodiment, a device which tests for the presence of specific species of bacterial or other procaryotic or eucaryotic contamination is provided. In another embodiment a device which indicates the presence of toxic material by either the generation or cessation or

diminution of bioluminescence generated by bioluminescent bacteria is provided.

The device in one embodiment comprises a sampling means; a sampling means washing means; a signal generating means, wherein said signal generating means is stably packaged; a means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means; and a sample and signal generating means mixing means.

By "biological material" is meant procaryotic, such as bacterial, or eucaryotic, such as molds, yeast, plant, animal and other eucaryotic organisms, cellular material wherein this material has associated with it ATP.

By "sampling means" is meant a device which allows one to obtain all of or a portion of a sample which may be present on a surface, in a solution or in an atmosphere to be tested. For example, the sampling means may be a swab comprising a shaft and an absorbent tip. The shaft of the sampling means may further comprise a venting means. The swab tip may be comprised of natural or synthetic materials so long as deposition of a sample thereto may occur. The material may be but is not limited to nylon, dacron, rayon, porex, absorbent polypropylene, absorbent polyethylene, nitrocellulose, cotton, wool, cellulose or sponges. In a preferred embodiment the swab shaft is hollow. Preferably the swab is provided for use in a pre-moistened form. This pre-moistening assists in the adsorption of biological or toxic material onto the surface of the sampling means. The moistening fluid may be a simple buffer or water. When the device is used with luciferase/luciferin as a signal generating means, the fluid may also act to release ATP from within cellular matter which may comprise the biological material adsorbed from a sampled surface. Fluids as are known in the art, for example Extralight® supplied by Analytical Luminescence Laboratory of San Diego, California may be used. Other fluids suitable for this purpose are set

forth in United States Patent 5,004,684, herein incorporated by reference. The sampling means may function through capillary action, for example a capillary tube or tubes. The sampling means may comprise a pipetting means. The sampling means may comprise a chamber which captures a sample of an atmosphere, such as the atmosphere present in an enclosed work space.

By "sampling means washing means" is meant a means which allows the removal of all or a part of a sample present on the "sampling means". For example, in one embodiment an upper housing means comprising a reservoir containing a fluid or dry substance in which the fluid or dry substance may be selectively released as desired, ordinarily to release a sample that has been obtained. The fluid may comprise the following: water, surfactants, salts, a neutralizing compound such as sodium thiosulfate. The fluid may be a bacterial or other procaryotic or eucaryotic nutrient-indicator medium as described in more detail below. Alternatively, a bacterial-regeneration fluid may be used to rehydrate lyophilized bacteria, when they are used as the signal-generating means. The dry substance may be but is not limited to a luciferase/luciferin composition, a lyophilized bacterial composition, a nutrient-indicator composition or other substance as appropriate, as set forth in more detail below. In an alternative embodiment, the upper housing means may contain a container such as, but not limited to, an ampule or a packet. The ampule or packet may contain a fluid or dry substance as described above. In an alternative embodiment the upper housing may contain two containers, both or either comprising, for example, but not limited to an ampule or packet, containing the same or different fluids or dry substances. In another embodiment a fluid may be directly contained in the upper housing and a container containing a fluid or dry substance be contained therein.

By "signal generating means" is meant a composition

which upon interaction with the sample may generate a signal which may be detected by human visual inspection or by detection in a device. Preferably, the signal comprises a change in the light emission or absorption characteristics of the sample. Most preferably the alteration of the luminescence (including amplitude, polarization, and other properties) of a signal is used. Even more preferably, a luciferase/luciferin composition is used to generate a signal. The luciferase/luciferin composition is preferably lyophilized and stably packaged. The luciferase/luciferin composition is highly sensitive and can detect the presence of as little as 10^{-16} moles of ATP. Other signal generating means such as bioluminescent bacteria and nutrient-indicator media may also be used. Other signal detection systems may be employed. Examples of such signal detection systems include, but are not limited to, spectrophotomers, colorimeters, luminometers, fluorometers, and devices that measure the decay of radioisotopes.

By "stably packaged" is meant that the signal generating means may be stored prior to use for prolonged periods of time, for example, over one year if stored at 2-8 degrees Centigrade, and still provide a signal upon activation. In one embodiment of the invention the signal generating means is stably packaged within a sealed glass ampule. The ampule may be a borosilicate glass, for example Pyrex®. It may be an "onionskin" type of glass ampule. In another embodiment a moisture proof foil or plastic packet may be used. More generally, any packaging which provides a good barrier to the loss or introduction of moisture may be used.

By "separation means" is meant a means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the mixing of sample and signal generating means is desired. By "separation means" is also meant that a breachable barrier exists between the signal generating

means and the sample. For example, the separation means may be a porous plastic or hydrophobic material filter, however, the porosity is not such that the sample would filter through without the application of a force, other than gravity, on the sample. The separation means may be a one way valve, a puncturable membrane, a temperature or chemical sensitive dissolvable membrane.

By "a sample and signal generating means mixing means" is meant a means of generating the force required to move the sample across the separation means set forth above. For example, a deformable lower section of a lower housing of the device, that is a region below the separation means and the sample, and which contains the signal generating means may be compressed and will allow for the passage of air or another gas from the lower section of the lower housing through the sample. Upon release of the pressure on the deformable lower section of the lower housing the pressure on the top of the sample will be greater than that below it. This pressure differential will drive the sample through the separation means. Concurrently, deformation of the lower section of the lower housing will have released the signal generating means from, for example, a glass ampule, thereby allowing mixing of the sample and signal generating means.

The method of using one embodiment of the device to test for the presence of ATP will be briefly described. An area to be tested for biological material is swabbed with the sampling means. Generally, approximately 100 square centimeters, *e.g.*, a 4" X 4" area, of surface is sampled. Because the swab is moistened with the moistening fluid, a portion of any biological material present on the surface should be adsorbed onto the swab. The sampling means is then sealably engaged onto an section of the lower housing. The sampling means washing means is activated. In one embodiment, this is done by bending a bulb containing the sample washing fluid, thereby breaking a seal and allowing the fluid to wash

down the hollow interior of the swab. The fluid washes the sample off of the swab. The fluid may also act to permeabilize any cells present in the sample, thereby releasing ATP contained therein. The sample is stably contained in the upper section of the lower housing of the device by the walls of the lower housing and the means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the generation of a signal is desired. Upon activation of the sample means mixing means, as described above, a signal may be generated. If luciferase/luciferin is the signal generating means used, a signal will be generated which is proportional to the amount of ATP, if any, present in the sample. The signal may be detected by a sampling device specifically adapted to be used with the sampling devices of this invention. The presence of ATP indicates that the area sampled contains biological material and may need to be cleaned, re-cleaned or sanitized.

Other specific applications of the invention are as follows: The testing of water samples to determine if they contain biological material. Testing of water samples to determine if they contain toxic material such as compounds which have bacteriocidal activity. Testing of samples for specific bacterial species (*e.g.*, Salmonella, *E. coli*, Listeria) or other procaryotic or eucaryotic organisms using a nutrient-indicator medium as the signal generating means.

By "toxic material" is meant material which has been associated with a detrimental effect on living organisms. By "detrimental effect" is meant a condition of decreased reproduction rate, less than optimal health, a disease or death. Compounds which activate "stress" inducible promoters of bacterial species are also included within this definition. Examples of toxic material include but are not limited to heavy metals, such as tin, lead, cadmium, mercury and chromium; organic chemicals, such as

pesticides and industrial waste products.

By "bacteriocidal activity" is meant a decrease or cessation in the rate of reproduction or the death of some or all of the bacteria exposed to a particular compound.

By "nutrient-indicator" medium is meant a medium which provides for the growth of specific target organisms but not others. The presence of the specific bacterial, mold or yeast species is made known by a signal generated on the basis of the cleavage of the nutrient-indicator releasing the indicator portion of the molecule by a specific enzyme, particular to a specific, bacterial, mold or yeast species.

The method of using one embodiment of the device to test for toxic material will be briefly described. A sample to be tested for toxic material, is collected, by a sampling means, for example a swab in the case of a surface or a pipette for a water sample. The sample is placed in the upper section of the lower housing of the device. The sample is washed off the sampling means by the sample means washing means, the washing means may contain additional reagents, for example bacterial reconstitution fluid. In the case of a liquid sample, washing may not be required but the addition of reagents may be desirable. A stable packaging means, for example, a glass ampule containing lyophilized bioluminescent bacteria may be provided in the lower section of the lower housing of the device. In an alternative embodiment, the bacteria could be present in an ampule in the upper housing means and which would be reconstituted there, for addition to the sample below. Upon mixing of the sample and the bacteria a signal may be generated if toxic material are present. The signal may be the increase or decrease of luminescence depending upon the bacterial system used as described in more detail below.

The method of using another embodiment of the device to test for the presence of specific species of bacteria, mold or yeast will be briefly described. A nutrient-

indicator medium as defined herein is provided in a stable packaging means, such as a glass ampule. A sample to be tested is added to the device, by swab or pipette or other sampling means. The sample is washed off the sampling means. The sample is mixed with the nutrient-indicator medium in the lower section of the lower housing of the device. If the particular species of bacteria responsive to the particular nutrient-indicator used are present a signal will be generated.

Other and further objects, features and advantages will be apparent from the following description of the presently preferred embodiments of the invention.

Detailed Description of the Invention

The drawings will first briefly be described.

Drawings:

Figure 1 is a cross-sectional illustration of one embodiment of the present invention.

Figure 2 is a schematic illustration of the components of one of the embodiments of the invention.

Figures 3A-3E illustrate alternative embodiments for providing means for packaging a fluid or dry substance in the device of the present invention.

Figures 4A-4D illustrate alternative embodiments for providing a sampling means to sample surfaces or fluids or gases.

Figures 5A-5E illustrate alternative embodiments for providing a separation means.

Figures 6A-6H illustrate the alternative embodiments for providing means for packaging a fluid or dry substance in the lower section of the device.

Turning in detail to the drawings:

Figure 1 illustrates an embodiment of the present invention which includes a top housing 10; an upper barrier means 11 between the top housing 10 and the upper section 13A of a lower housing 14C; a means 12 for breaking the upper barrier means 11; a sampling means 15A comprised of a shaft 15B and a surface sampling means 15C;

a sampling means engaging means 13B which may optionally fit inside of the upper section 14A of the lower housing 14C; a separation means 17 separating the upper section 14A of lower housing 14C from a lower section 14B of the lower housing 14C; a signal means packaging means 18. In Figure 1, the top housing 10 and upper barrier 11 define a chamber 19 which optionally may contain a sealed or partially sealed container as shown in Figure 3.

Figure 2 illustrates a top housing 20, an upper barrier means 21 between the top housing 20 and the upper section 22 of a lower housing 27. The top housing 20 and upper barrier means 21 define a chamber 28 which optionally may contain a sealed or partially sealed container as shown in Figure 3. A sampling means 23. A separation means 24 separating the upper section 22 of lower housing 27 from a lower section 25 of the lower housing 27. A signal means packaging means 26.

Figure 3 illustrates alternative embodiments for providing means for packaging a fluid or dry substance in the chamber 28. Figure 3A illustrates a container 29 within chamber 28. Figure 3B illustrates a fluid 30 directly contained by the walls of the top housing 20 and upper barrier means 21. Figure 3C illustrates two containers 29A and 29B containing the same or different fluids. Figure 3D illustrates two containers 29C and 29A, 29C containing a dry substance and 29A a fluid. Figure 3E illustrates a fluid 30 directly contained by the walls of the top housing 20 and upper barrier means 21 and a container 29C containing a dry substance.

Figure 4 illustrates alternative embodiments for providing a sampling means to sample surfaces or fluids or gases. Figure 4A illustrates a shaft 40A with a central channel 40B and channels 40C extending into a surface sampling means 41. Figure 4B illustrates a solid shaft 42 attached to a surface sampling means 43. Figure 4C illustrates a pipetting means 44 for sampling fluids. Figure 4D illustrates that a separate sampling means, such

as a pipette 45 may be used.

Figure 5 illustrates alternative embodiments for providing a separation means. Figure 5A illustrates a semi-porous filter element 50. Figure 5B illustrates a one-way valving means. Figure 5C illustrates a puncturable membrane 52 and a puncturing device 53. Figure 5D illustrates a porous separation means 54. Figure 5E illustrates a temperature or solvent dissolvable membrane 55.

Figures 6A to 6H illustrate alternative embodiments for providing means for packaging a fluid or dry substance in a chamber 60 of the lower section 25 of lower housing 27. Figure 6A illustrates a container 61 containing a fluid or dry substance within chamber 60. Figure 6B illustrates a plastic or foil packet 62 containing a fluid or dry substance within chamber 60. Figure 6C illustrates a dry substance 63 directly contained within chamber 60. Figure 6D illustrates a fluid 64 directly contained within chamber 60. Figure 6E illustrates an ampule 65 within an ampule 66 or a packet 66 within a packet 66. The ampule or packet 65 may contain a fluid or dry substance. The ampule or packet 66 may contain a fluid or dry substance. The fluid or dry substance in ampule or packet 65 or 66 may be the same or different. Figure 6F illustrates an ampule 67 and an ampule 68 or a packet 67 and a packet 68. The ampule or packet 67 may contain a fluid or dry substance. The ampule or packet 68 may contain a fluid or dry substance. The fluid or dry substance in ampule or packet 67 or 68 may be the same or different. Figure 6G illustrates a fluid 69B directly contained in chamber 60 and a container 69A containing a fluid or dry substance. Figure 6H illustrates an ampule or packet 70 containing a first substance 70A and a second substance 70B.

It will be readily apparent to one skilled in the art that various substitutions and modification may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The present invention, in one embodiment, describes a device and methods for the rapid and convenient testing of surfaces, solutions or gases for biological contamination by assaying for the presence of adenosine triphosphate ("ATP").

As described briefly above, the "sampling means washing means" may comprise for example, a reservoir containing a fluid in which the fluid may be selectively released after a sample has been obtained. The fluid may comprise the following: water, surfactants, salts, a neutralizing compound, such as sodium thiosulfate. More specifically, a suitable formulation is as follows: 50 mM Trizma base, 0.5 mM $MgCl_2$, 0.5% w/v Tween 80, 0.01 g/L Sodium Thiosulfate, pH to about 7.7 to 8.0 with, for example, acetic acid, a preservative, such as Rohm and Haas' Proclin 300 may be added to about 10-40 ppm, preferably 15 ppm. Further, an additional preservative, such as sodium Azide, may be added to 0.02% w/v.

Also as briefly described above a "signal generating means" such as a luciferase/luciferin composition may be used. Specifically, a suitable luciferase/luciferin composition comprises the following: Luciferase at a concentration of from about 50-100 μg/ml (the higher end of this range is preferable); Luciferin at a concentration of from about 0.1-4.0 mg/ml (the middle to high end of this range is preferable); EDTA at a concentration of from about 0.1 - 2.0 mM, (the middle of this range is preferable); DTT at a concentration of from about 2.0 - 4.0 mM (the middle of this range is preferable); BSA at about 5.0 - 50.0 mg/ml, (the middle of this range is preferred); Trizma at about 25 - 75 mM, (the middle of this range is preferred); $MgCl_2$ at a concentration of about 25.0 - 100.0 mM (the middle of this range is preferred). Other buffer compositions may be suitably used. A preferred composition would be as follows: Luciferase 76 μg/ml, BSA 25 mg/ml, Luciferin 2 mg/ml, EDTA 1mM, DTT 3 mM, Trizma 50 mM, $MgCl_2$ 50 mM. The

luciferase/luciferin composition is preferably lyophilized and stably packaged. Specifically, preparation of a luciferase/luciferin composition suitable for use in one embodiment of this invention may be done as follows: a 25 μl aliquot of the "luciferase/luciferin cocktail", (the luciferase/luciferin cocktail compositions are obtainable, for example, from Sigma chemical corporation or JBL or Boehringer Mannheim; by "cocktail" is meant the luciferin and luciferase and other elements that allow the system to produce a signal upon exposure to ATP), is deposited and frozen in a thin-walled glass onion-skin tube ("OST"). The aliquot is lyophilized to a moisture content of about less than 4%. The OST is then removed from the lyophilizer to a dry environment where the ends of the OST are melted in a high temperature flame, hermetically sealing the material inside the glass ampule.

Other specific applications of the invention are as follows:

(1) The testing of water samples to determine if they contain biological material. The procedure utilized to test for biological material in a water sample would be similar to the procedure used to test a surface, with the difference being that the sample tested is a liquid.

(2) The testing of samples for toxic material or compounds having bacteriocidal activity. Two suitable procedures are as follows: First, bacteria which produce bacterial luciferase, "bioluminescent bacteria" would be lyophilized as is known in the art. These bioluminescent bacteria would be placed into a stable packaging means in the device of the present invention. A sample would be added, as described elsewhere regarding the operation of the device. Upon reconstitution these bioluminescent bacteria will "bioluminesce", thereby indicating their viability. Upon addition of a sample containing a toxic material of a particular threshold concentration the bacteria will cease exhibiting bioluminescence, indicating the presence of a toxic material. Bacterial reagents as

are known in the art, for example the Microtox® reagent provided by Microbics Corporation of Carlsbad, California may be utilized. Ecotoxicology Monitoring, Section 3, Chapters 13-23, Mervyn Richardson Editor, (1993) is hereby incorporated by reference.

Alternatively, the luciferase gene may be cloned behind a promoter that is activated by various substances, for example, heavy metals. Upon the addition of a sample containing activating toxic material, for example, heavy metals, luciferase will be produced providing a detectable signal. Construction of a bacterial plasmid may be done as is known in the art and as set forth in Molecular Cloning, a Laboratory Manual Second Edition, Sambrook et al., incorporated herein by reference. Heavy metal inducible promoters and/or bacterial bioluminescent sensor strategies are disclosed in the following publications: Abstracts of the American Society for Microbiology, 93 general Meeting 16-20 May 1993, Atlanta Georgia; Abstract No. Q-95 Development of Bioluminescent Biosensors for Copper and Mercury, Gangolli et al.; Abstract No. Q-98 Biosensors Utilizing Promoterless Xenorhabdus luminescens lux for Detection of Mercury, Other Divalent Cations, and Selected Organics, Taylor et al.; Abstract No. Q-99 Transfer of an AlgD-lux Reporter Plasmid to Environmental Bacterial Strains and Demonstration of Bioluminescence in Relation to Factors Known to Influence Exopolysaccharide Synthesis, Rice et al.; Pollutant Detection by Induction of a Heat Shock Promoter-lux Gene Fusion in Escherichia coli K12, Van Dyk et al.; Stuart, "A 12-Base-Pair DNA Motif That is Repeated Several Times in Metallothionein Gene Promoters Confers Metal Regulation to a Heterologous Gene," 81 *Proc. Natl. Acad. Sci. USA* 7318-7322, 1984, all of the above incorporated by reference herein.

(3) In another embodiment of the invention the testing of samples for specific procaryotic bacterial species (*e.g.*, Salmonella, *E. coli*, Listeria) or eucaryotic organisms such as yeast is performed. In this

aspect of the invention, the signal generating means comprises a nutrient-indicator media, such as Colilert® nutrient-indicator media, available from IDEXX Laboratories of Westbrook, Maine. United States Patent 4,925,789 detailing the composition and use of nutrient-indicator media is incorporated herein by reference. A liquid sample is obtained with for example a pipette. The sample is mixed with the nutrient-indicator media. The whole device is incubated at a required temperature, dependent on the microorganism which one wishes to detect, for a specified time. For example, 35 degrees Centigrade for *E. coli*. If specific species of target bacteria are present a signal will be generated. More specifically, *E. coli* contain the enzyme β-glucorindase, while coliform bacteria contain the enzyme β-galactosidase. In the presence of the nutrient-indicator 4-methylumbelliferyl-β-D-glucuronide (MUG), *E. coli* will metabolize this compound, releasing an indicator portion, which when cleaved fluoresces when excited by an ultraviolet light source. Alternatively, coliform, in the presence of the nutrient-indicator o-nitrophenyl-β-D-galactoside (ONPG) will metabolize this compound, releasing an indicator portion, which when cleaved colors a sample yellow.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All such patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods, procedures, treatments, and devices described herein are presently representative of preferred embodiments, are exemplary and are not intended as limitations on the scope of the invention. Changes

therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

CLAIMS

What is claimed is:

1. A device comprising:

(a) a sampling means;

(b) a sampling means washing means wherein said sampling means is contained within a housing means providing protection from pre-testing contamination for said sampling means;

(c) a signal generating means, wherein said signal generating means is stably packaged;

(d) a separation means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the mixing of sample and signal generating means is desired; and

(e) a sample and signal generating means mixing means, wherein said elements (c)-(e) are contained in a lower housing, said lower housing divided into an upper and lower section by element (d).

2. The device of claim 1, wherein said sampling means is a swab.

3. The device of claim 1, wherein said sampling means is a pipette.

4. The device of claim 1, wherein said sampling means is a capillary tube.

5. The device of claim 1, wherein said sampling means washing means is a fluid which may be released from said upper housing containing said fluid.

6. The device of claim 1, wherein said signal generating means is a luciferase/luciferin composition.

7. The device of claim 6, wherein said luciferase/luciferin composition comprises: Luciferase at a concentration of from about 50-100 μg/ml; Luciferin at a concentration of from about 0.1-4.0 mg/ml and a suitable buffer composition.

8. The device of claim 6, wherein said luciferase/luciferin composition comprises: Luciferase at

a concentration of from about 50-100 μg/ml; Luciferin at a concentration of from about 0.1-4.0 mg/ml; EDTA at a concentration of from about 0.1 - 2.0 mM; DTT at a concentration of from about 2.0 - 4.0 mM; BSA at about 5.0 - 50.0 mg/ml; Trizma at about 25 - 75 mM; $MgCl_2$ at a concentration of about 25.0 - 100.0 mM.

9. The device of claim 6, wherein said signal luciferase/luciferin composition is stably packaged in a moisture proof container.

10. The device of claim 6, wherein said signal luciferase/luciferin composition is stably packaged in a glass ampule.

11. The device of claim 1, wherein said signal generating means is a nutrient-indicator composition.

12. The device of claim 10, wherein said nutrient-indicator composition is Colilert®.

13. The device of claim 10, wherein said nutrient-indicator composition is stably packaged in a glass ampule.

14. The device of claim 1, wherein said signal generating means are bioluminescent bacteria.

15. The device of claim 13, wherein said bioluminescent bacteria produce a signal upon exposure to a toxic material.

16. The device of claim 14, wherein said signal produced upon exposure to a toxic material is bioluminescence.

17. The device of claim 14, wherein said signal produced upon exposure to a toxic material is the cessation of or decrease of bioluminescence.

18. The device of claim 1, wherein said separation means is a semi-porous filter.

19. The device of claim 1, wherein said separation means is a porous filter.

20. The device of claim 1, wherein said separation means is a puncturable membrane.

21. The device of claim 1, wherein said separation

means is a temperature dissolvable membrane.

22. The device of claim 1, wherein said separation means is a chemical dissolvable membrane.

23. The device of claim 1, wherein said sample and signal generating means mixing means, comprises said lower section of said lower housing, wherein said lower section of said lower housing is deformable and upon compression allows for the creation of a pressure differential which upon release of the housing drives a sample washed from said sampling means by said sample means washing means into said lower section of said lower housing.

24. A device comprising:

(a) a receptacle for containing a fluid;

(b) said receptacle further comprising a breakable shaft which upon breakage exposes an orifice through which the fluid may flow;

(c) said receptacle adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(d) a swab means comprising a hollow shaft and an absorbent tip wherein said hollow shaft is in communication with said orifice in said receptacle;

(e) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(f) a moisture proof sealed vessel containing a lyophilized luciferase/luciferin composition contained in said lower section of said lower housing.

25. A device comprising:

(a) a pipette means wherein said pipette means is adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(b) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(c) a moisture proof sealed vessel containing lyophilized bioluminescent bacteria contained in said lower section of said lower housing, wherein said lower section of said lower housing further comprises a bacterial regeneration fluid.

26. The device of claim 25 wherein said bioluminescent bacteria are Microtox® bioluminescent bacteria.

27. A device comprising:

(a) a receptacle for containing a fluid;

(b) said receptacle further comprising a breakable shaft which upon breakage exposes an orifice through which the fluid may flow;

(c) said receptacle adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(d) a swab means comprising a hollow shaft and an absorbent tip wherein said hollow shaft is in communication with said orifice in said receptacle;

(e) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(f) a moisture proof sealed vessel containing nutrient-indicator media contained in said lower section of said lower housing.

28. The device of claim 26 wherein said nutrient-indicator media is Colilert® nutrient indicator media.

29. A device comprising:

(a) a pipette means wherein said pipette means is adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(b) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(c) a moisture proof sealed vessel containing

nutrient-indicator media contained in said lower section of said lower housing.

30. The device of claim 28 wherein said nutrient-indicator media is Colilert® nutrient indicator media.

31. A method for testing for the presence of biological matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with a signal generating means allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

32. A method for testing for the presence of biological matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with a nutrient-indicator composition, allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

33. A method for testing for the presence of toxic matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with bioluminescent bacteria, allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

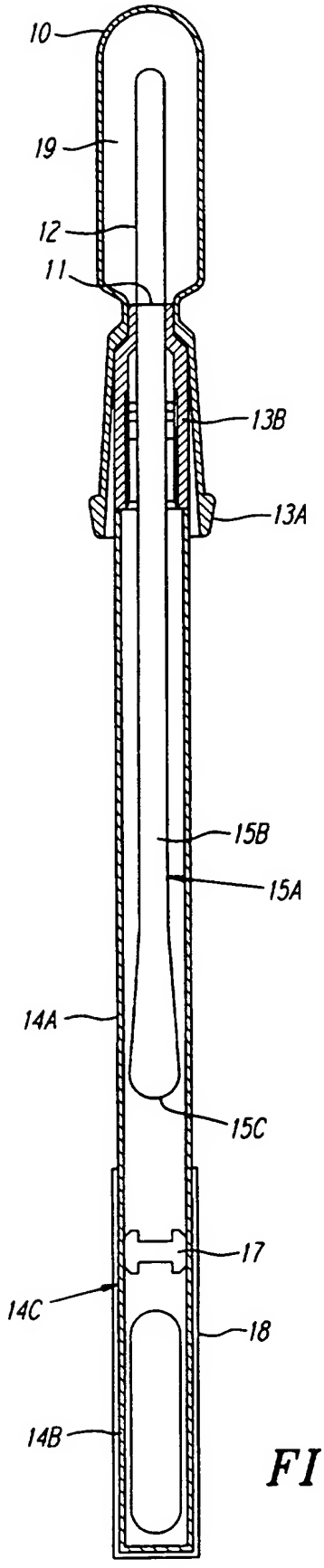

FIG. 1

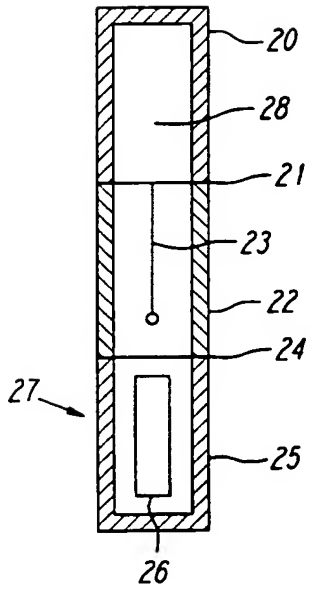

FIG. 2

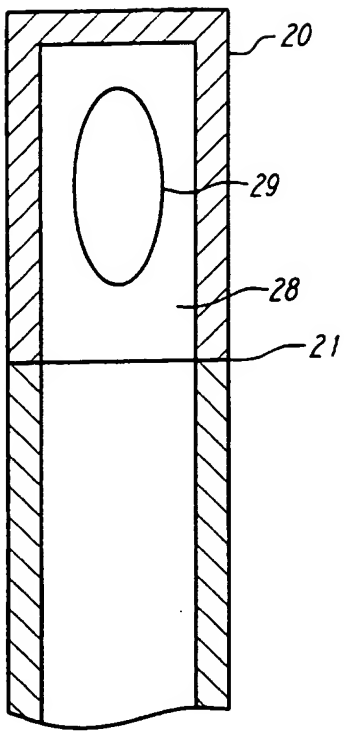

FIG. 3A

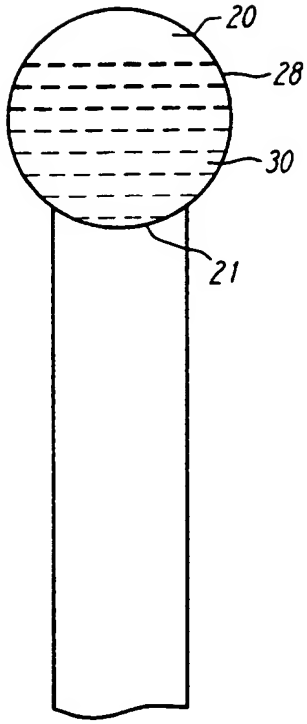

FIG. 3B

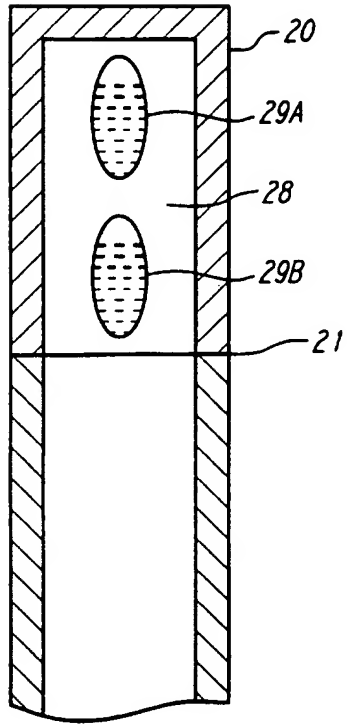

FIG. 3C

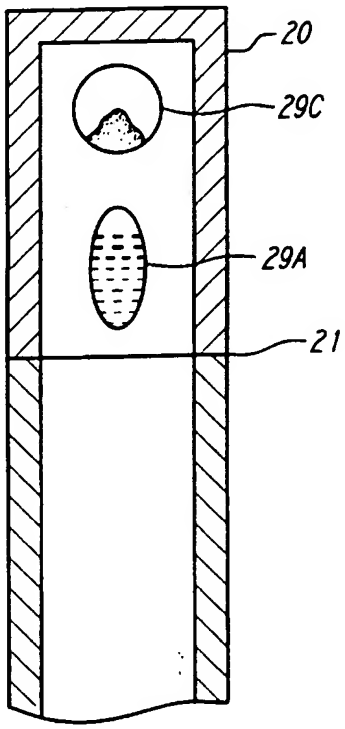

FIG. 3D

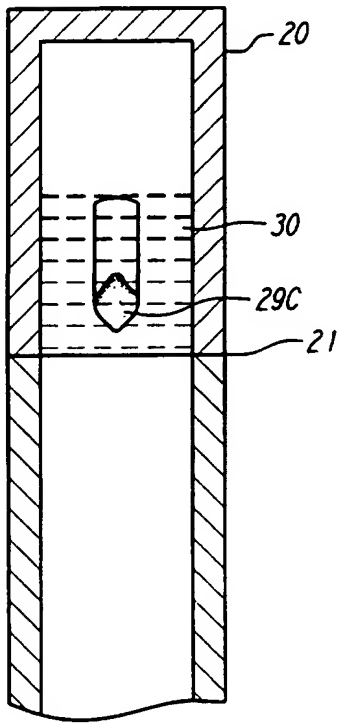

FIG. 3E

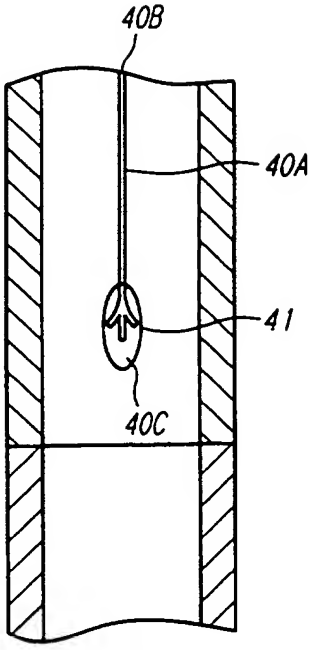

FIG. 4A

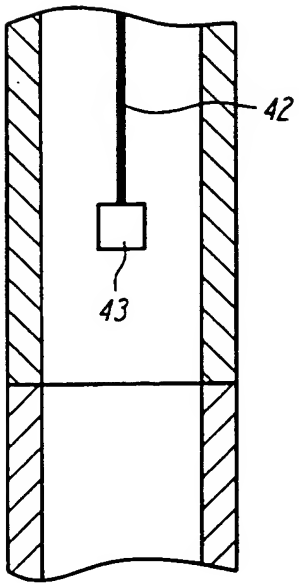

FIG. 4B

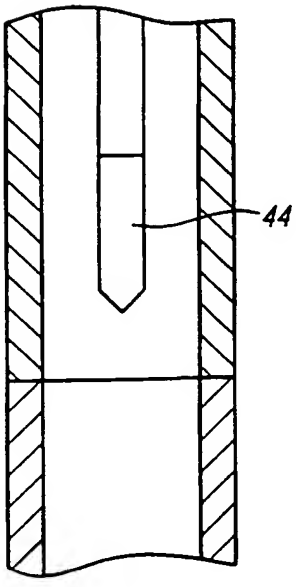

FIG. 4C

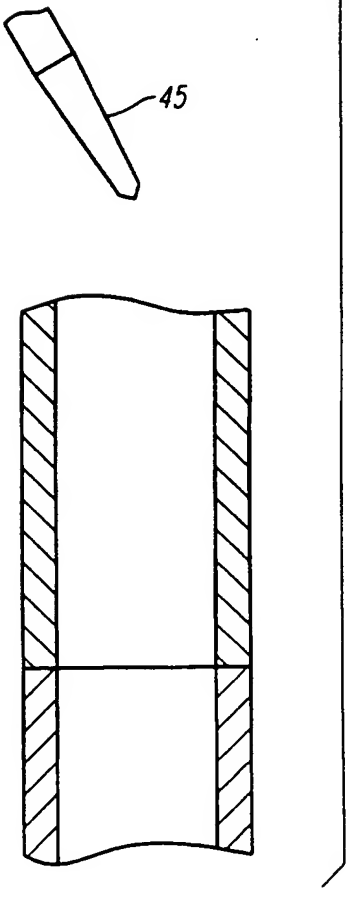

FIG. 4D

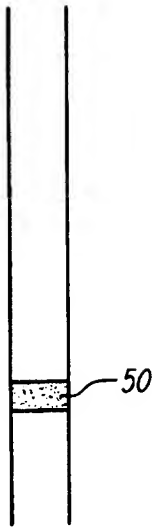

FIG. 5A

FIG. 5B

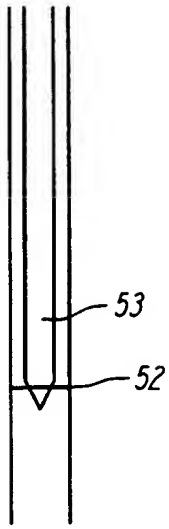

FIG. 5C

FIG. 5D

FIG. 5E

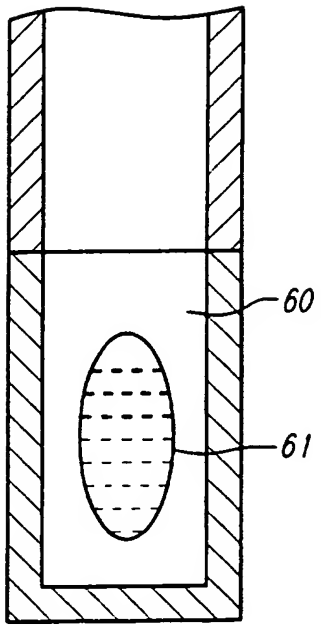

FIG. 6A

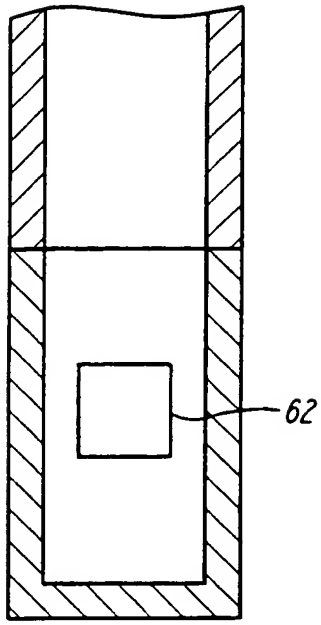

FIG. 6B

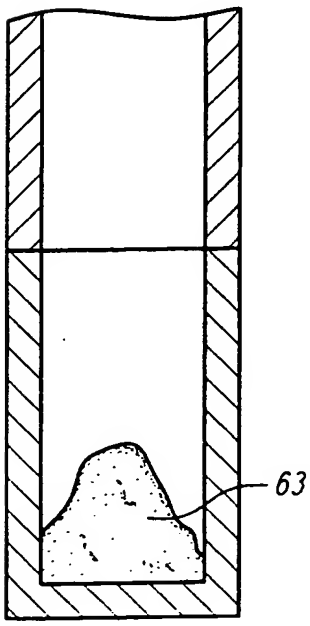

FIG. 6C

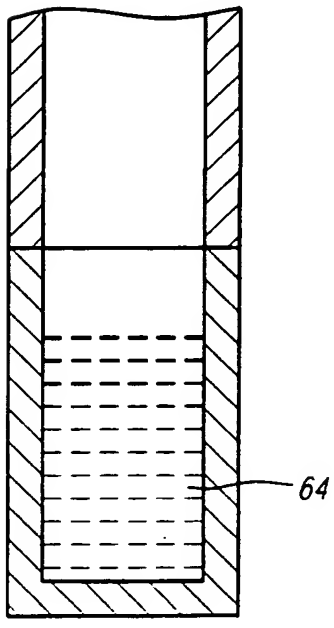

FIG. 6D

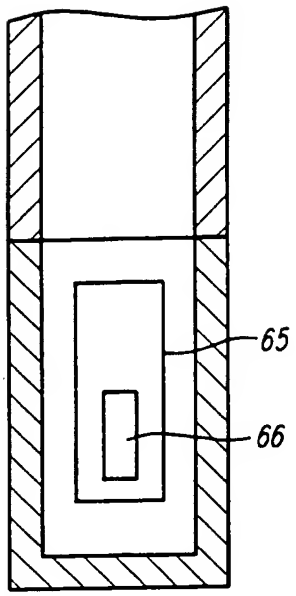

FIG. 6E

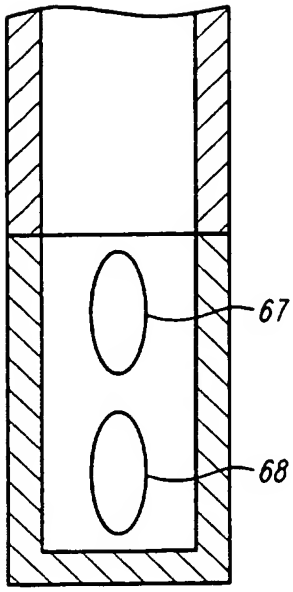

FIG. 6F

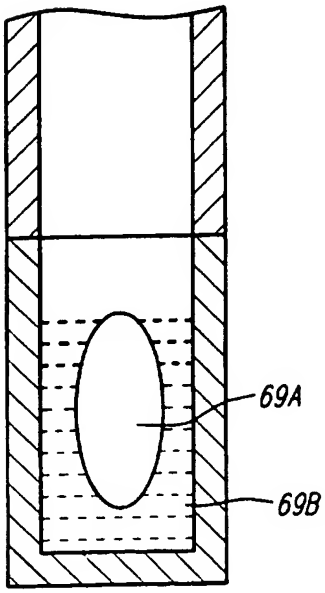

FIG. 6G

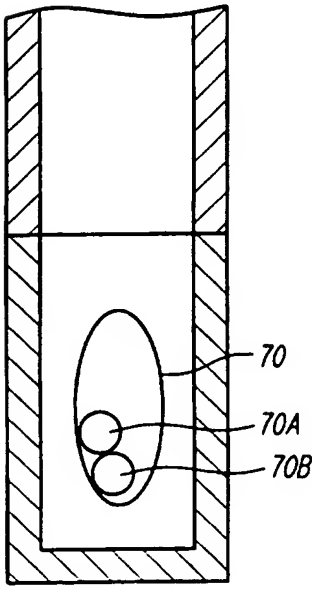

FIG. 6H

INTERNATIONAL SEARCH REPORT

Inte[illegible]onal Application No
PCT/US 95/13147

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/76 G01N1/02 B01L3/00 C12M1/30 A61B10/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L C12M G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 12421 (NASON) 24 June 1993	1,2,5, 18,19, 23,31
Y	see page 7, paragraph 1 - paragraph 2; figures 1,2	3,4, 6-17, 20-22, 24,27, 32,33
	see page 12, last paragraph - page 13, paragraph 1; figure 8	
	see page 13, paragraph 2; figures 10-12	
	see page 15, paragraph 1 - paragraph 3; figure 13	

	-/--	

[X] Further documents are listed in the continuation of box C.

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1

Date of the actual completion of the international search
15 March 1996

Date of mailing of the international search report
27.03.96

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	see page 11, paragraph 3; figure 4E	4
Y	US,A,5 188 965 (DIFCO) 23 February 1993 see column 1, line 59 - column 2, line 15 see column 3, line 9 - line 61 see column 5, line 45 - line 66	6-10,24
Y	US,A,4 207 394 (ALDRIDGE) 10 June 1980 see column 5, line 6 - line 22 see abstract	11-13, 27,29,32
Y	GB,A,2 005 018 (BECKMAN INSTRUMENTS) 11 April 1979 see abstract; claims 1,2,16,17	14-17, 25,33
Y	PATENT ABSTRACTS OF JAPAN vol. 14 no. 306 (C-0735) ,3 July 1990 & JP,A,02 104273 (RICOH) 17 April 1990, see abstract	21
Y	US,A,5 047 044 (SMITH) 10 September 1991 see column 33, line 7 - line 19; figure 20	22
P,X	WO,A,95 25948 (CELCIS) 28 September 1995 see claims 1-5; figure 4	1,2,6-10
A	US,A,5 313 959 (MONTHONY) 24 May 1994 see column 4, line 12 - line 21 see column 4, line 22 - line 55 see column 5, line 40 - column 7, line 50; figure 9	1-33
A	WO,A,93 00994 (AMERSHAM) 21 January 1993 see page 3, line 26 - page 4, line 24; figure 1	

1

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/US 95/13147

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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information on patent family members

Inter ial Application No
PCT/US 95/13147

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9300994		US-A- 5449494	12-09-95

CORRECTED VERSION*

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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(71) Applicant: IDEXX LABORATORIES, INC. [US/US]; One Idexx Drive, Westbrook, ME 04092 (US).

(72) Inventors: EHRENFELD, Elizabeth; 6 Shoreline Drive, Falmouth, ME 04105 (US). CARPENTER, Charles; 175 Holmes Road, Scarborough, ME 04074 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

(81) Designated States: CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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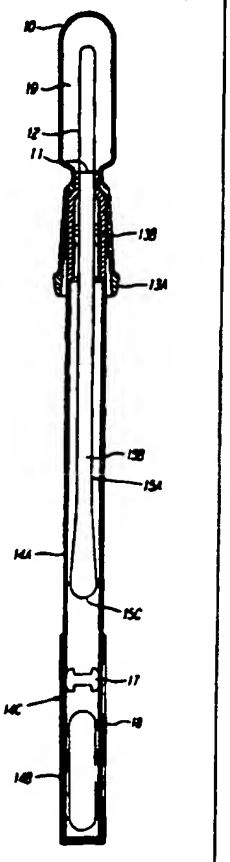
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SELF-CONTAINED SIGNAL GENERATING SAMPLING DEVICE AND METHODS OF USE OF SAME

(57) Abstract

A self-contained signal generating device and methods for use of such a device are provided. The device and methods may detect the presence of biological or toxic materials and may utilize, among other compositions, a luciferin/luciferase composition, nutrient-indictor media or bioluminescent bacteria to provide a signal indicative of the presence of biological or toxic material.



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DESCRIPTION

Self-Contained Signal Generating Sampling Device and Methods of Use of Same

Background of the Invention

This invention relates in general to a device and methods for generating a signal in response to the testing of a sample which may contain biological material or toxic material wherein the device generating the signal is self-contained.

The use of sampling devices in various fields is known. For example, the sampling for contamination of food processing facilities; the sampling of contamination of the environment by heavy metals such as lead or cadmium; the collection of specimens from a patient to test for microorganism infection.

Generally, the devices and methods previously used required various inconvenient steps. For example, batch preparation of a chemical which would provide a desired signal upon contact with a signal-generating sample, followed by pipetting of the batch preparation into multiple tubes. This preparation might take place in a food processing facility where contamination of the food being processed by foreign substances, such as a signal-generating chemical is a major concern. Often, for reasons of personnel turnover or otherwise, the personnel doing the sampling may lack significant experience preparing chemical formulations, thereby introducing the risk of error in preparation of the signal-generating composition. Other devices have required shipment back to laboratories for further processing and/or analysis of samples obtained. For example, food processing facilities often use bacterial plating and counting techniques which could take 24-48 hours to provide results. Environmental sampling for biological contamination often takes up to five days.

Some devices have attempted tc overcome these problems by designing a self-contained testing unit. However, when particular signal generating chemicals such as luciferase/luciferin are used their design is believed not to offer the same degree of stability or ease of use of the luciferase/luciferin as in the present invention. Further, due to the sensitive nature of the test for biological contamination, contamination of the testing device prior to use must be avoided. The present invention provides for the protection of a sampling means prior to use, whereas other devices leave the sampling means relatively accessible to pre-test contamination.

The demand for a self-contained, easily used, stable, and storable testing device, yielding immediate results is greater than ever. This demand has occurred in light of increased consumer and regulatory pressure, for example, in response to recent deaths and illness caused by contamination of beef at fast food restaurants and incidents involving contaminated cheese and milk products. The device of the present invention fulfills this need.

Summary of the Invention

This invention in one aspect features a device which allows for the rapid and convenient testing of surfaces, solutions or atmospheres for biological contamination by assaying for the presence of adenosine triphosphate ("ATP"). ATP is assayed for because it is a ubiquitous cellular component in both procaryotes and eucaryotes. For example, in one application, the device will allow for the testing of surfaces in, for example, food processing factories, supermarkets and fast food restaurants to determine if such surfaces contain biological material. In another embodiment, a device which tests for the presence of specific species of bacterial or other procaryotic or eucaryotic contamination is provided. In another embodiment a device which indicates the presence of toxic material by either the generation or cessation cr

diminution of bioluminescence generated by bioluminescent bacteria is provided.

The device in one embodiment comprises a sampling means; a sampling means washing means; a signal generating means, wherein said signal generating means is stably packaged; a means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means; and a sample and signal generating means mixing means.

By "biological material" is meant procaryotic, such as bacterial, or eucaryotic, such as molds, yeast, plant, animal and other eucaryotic organisms, cellular material wherein this material has associated with it ATP.

By "sampling means" is meant a device which allows one to obtain all of or a portion of a sample which may be present on a surface, in a solution or in an atmosphere to be tested. For example, the sampling means may be a swab comprising a shaft and an absorbent tip. The shaft of the sampling means may further comprise a venting means. The swab tip may be comprised of natural or synthetic materials so long as deposition of a sample thereto may occur. The material may be but is not limited to nylon, dacron, rayon, porex, absorbent polypropylene, absorbent polyethylene, nitrocellulose, cotton, wool, cellulose or sponges. In a preferred embodiment the swab shaft is hollow. Preferably the swab is provided for use in a pre-moistened form. This pre-moistening assists in the adsorption of biological or toxic material onto the surface of the sampling means. The moistening fluid may be a simple buffer or water. When the device is used with luciferase/luciferin as a signal generating means, the fluid may also act to release ATP from within cellular matter which may comprise the biological material adsorbed from a sampled surface. Fluids as are known in the art, for example Extralight© supplied by Analytical Luminescence Laboratory of San Diego, California may be used. Other fluids suitable for this purpose are set

forth in United States Patent 5,004,684, herein incorporated by reference. The sampling means may function through capillary action, for example a capillary tube or tubes. The sampling means may comprise a pipetting means. The sampling means may comprise a chamber which captures a sample of an atmosphere, such as the atmosphere present in an enclosed work space.

By "sampling means washing means" is meant a means which allows the removal of all or a part of a sample present on the "sampling means". For example, in one embodiment an upper housing means comprising a reservoir containing a fluid or dry substance in which the fluid or dry substance may be selectively released as desired, ordinarily to release a sample that has been obtained. The fluid may comprise the following: water, surfactants, salts, a neutralizing compound such as sodium thiosulfate. The fluid may be a bacterial or other procaryotic or eucaryotic nutrient-indicator medium as described in more detail below. Alternatively, a bacterial-regeneration fluid may be used to rehydrate lyophilized bacteria, when they are used as the signal-generating means. The dry substance may be but is not limited to a luciferase/luciferin composition, a lyophilized bacterial composition, a nutrient-indicator composition or other substance as appropriate, as set forth in more detail below. In an alternative embodiment, the upper housing means may contain a container such as, but not limited to, an ampule or a packet. The ampule or packet may contain a fluid or dry substance as described above. In an alternative embodiment the upper housing may contain two containers, both or either comprising, for example, but not limited to an ampule or packet, containing the same or different fluids or dry substances. In another embodiment a fluid may be directly contained in the upper housing and a container containing a fluid or dry substance be contained therein.

By "signal generating means" is meant a composition which upon interaction with the sample may generate a signal which may be detected by human visual inspection or by detection in a device. Preferably, the signal comprises a change in the light emission or absorption characteristics of the sample. Most preferably the alteration of the luminescence (including amplitude, polarization, and other properties) of a signal is used. Even more preferably, a luciferase/luciferin composition is used to generate a signal. The luciferase/luciferin composition is preferably lyophilized and stably packaged. The luciferase/luciferin composition is highly sensitive and can detect the presence of as little as 10^{-16} moles of ATP. Other signal generating means such as bioluminescent bacteria and nutrient-indicator media may also be used. Other signal detection systems may be employed. Examples of such signal detection systems include, but are not limited to, spectrophotomers, colorimeters, luminometers, fluorometers, and devices that measure the decay of radioisotopes.

By "stably packaged" is meant that the signal generating means may be stored prior to use for prolonged periods of time, for example, over one year if stored at 2-8 degrees Centigrade, and still provide a signal upon activation. In one embodiment of the invention the signal generating means is stably packaged within a sealed glass ampule. The ampule may be a borosilicate glass, for example Pyrex®. It may be an "onionskin" type of glass ampule. In another embodiment a moisture proof foil or plastic packet may be used. More generally, any packaging which provides a good barrier to the loss or introduction of moisture may be used.

By "separation means" is meant a means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the mixing of sample and signal generating means is desired. By "separation means" is also meant that a

breachable barrier exists between the signal generating means and the sample. For example, the separation means may be a porous plastic or hydrophobic material filter, however, the porosity is not such that the sample would filter through without the application of a force, other than gravity, on the sample. The separation means may be a one way valve, a puncturable membrane, a temperature or chemical sensitive dissolvable membrane.

By "a sample and signal generating means mixing means" is meant a means of generating the force required to move the sample across the separation means set forth above. For example, a deformable lower section of a lower housing of the device, that is a region below the separation means and the sample, and which contains the signal generating means may be compressed and will allow for the passage of air or another gas from the lower section of the lower housing through the sample. Upon release of the pressure on the deformable lower section of the lower housing the pressure on the top of the sample will be greater than that below it. This pressure differential will drive the sample through the separation means. Concurrently, deformation of the lower section of the lower housing will have released the signal generating means from, for example, a glass ampule, thereby allowing mixing of the sample and signal generating means.

The method of using one embodiment of the device to test for the presence of ATP will be briefly described. An area to be tested for biological material is swabbed with the sampling means. Generally, approximately 100 square centimeters, *e.g.*, a 4" X 4" area, of surface is sampled. Because the swab is moistened with the moistening fluid, a portion of any biological material present on the surface should be adsorbed onto the swab. The sampling means is then sealably engaged onto an section of the lower housing. The sampling means washing means is activated. In one embodiment, this is done by bending a bulb containing the sample washing fluid, thereby breaking

a seal and allowing the fluid to wash down the hollow interior of the swab. The fluid washes the sample off of the swab. The fluid may also act to permeabilize any cells present in the sample, thereby releasing ATP contained therein. The sample is stably contained in the upper section of the lower housing of the device by the walls of the lower housing and the means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the generation of a signal is desired. Upon activation of the sample means mixing means, as described above, a signal may be generated. If luciferase/luciferin is the signal generating means used, a signal will be generated which is proportional to the amount of ATP, if any, present in the sample. The signal may be detected by a sampling device specifically adapted to be used with the sampling devices of this invention. The presence of ATP indicates that the area sampled contains biological material and may need to be cleaned, re-cleaned or sanitized.

Other specific applications of the invention are as follows: The testing of water samples to determine if they contain biological material. Testing of water samples to determine if they contain toxic material such as compounds which have bacteriocidal activity. Testing of samples for specific bacterial species (*e.g.*, Salmonella, *E. coli*, Listeria) or other procaryotic or eucaryotic organisms using a nutrient-indicator medium as the signal generating means.

By "toxic material" is meant material which has been associated with a detrimental effect on living organisms. By "detrimental effect" is meant a condition of decreased reproduction rate, less than optimal health, a disease or death. Compounds which activate "stress" inducible promoters of bacterial species are also included within this definition. Examples of toxic material include but

are not limited to heavy metals, such as tin, lead, cadmium, mercury and chromium; organic chemicals, such as pesticides and industrial waste products.

By "bacteriocidal activity" is meant a decrease or cessation in the rate of reproduction or the death of some or all of the bacteria exposed to a particular compound.

By "nutrient-indicator" medium is meant a medium which provides for the growth of specific target organisms but not others. The presence of the specific bacterial, mold or yeast species is made known by a signal generated on the basis of the cleavage of the nutrient-indicator releasing the indicator portion of the molecule by a specific enzyme, particular to a specific, bacterial, mold or yeast species.

The method of using one embodiment of the device to test for toxic material will be briefly described. A sample to be tested for toxic material, is collected, by a sampling means, for example a swab in the case of a surface or a pipette for a water sample. The sample is placed in the upper section of the lower housing of the device. The sample is washed off the sampling means by the sample means washing means, the washing means may contain additional reagents, for example bacterial reconstitution fluid. In the case of a liquid sample, washing may not be required but the addition of reagents may be desirable. A stable packaging means, for example, a glass ampule containing lyophilized bioluminescent bacteria may be provided in the lower section of the lower housing of the device. In an alternative embodiment, the bacteria could be present in an ampule in the upper housing means and which would be reconstituted there, for addition to the sample below. Upon mixing of the sample and the bacteria a signal may be generated if toxic material are present. The signal may be the increase or decrease of luminescence depending upon the bacterial system used as described in more detail below.

The method of using another embodiment of the device to test for the presence of specific species of bacteria, mold or yeast will be briefly described. A nutrient-indicator medium as defined herein is provided in a stable packaging means, such as a glass ampule. A sample to be tested is added to the device, by swab or pipette or other sampling means. The sample is washed off the sampling means. The sample is mixed with the nutrient-indicator medium in the lower section of the lower housing of the device. If the particular species of bacteria responsive to the particular nutrient-indicator used are present a signal will be generated.

Other and further objects, features and advantages will be apparent from the following description of the presently preferred embodiments of the invention.

Detailed Description of the Invention

The drawings will first briefly be described.

Drawings:

Figure 1 is a cross-sectional illustration of one embodiment of the present invention.

Figure 2 is a schematic illustration of the components of one of the embodiments of the invention.

Figures 3A-3E illustrate alternative embodiments for providing means for packaging a fluid or dry substance in the device of the present invention.

Figures 4A-4D illustrate alternative embodiments for providing a sampling means to sample surfaces or fluids or gases.

Figures 5A-5E illustrate alternative embodiments for providing a separation means.

Figures 6A-6H illustrate the alternative embodiments for providing means for packaging a fluid or dry substance in the lower section of the device.

Turning in detail to the drawings:

Figure 1 illustrates an embodiment of the present invention which includes a top housing 10; an upper barrier means 11 between the top housing 10 and the upper section 13A of a lower housing 14C; a means 12 for breaking the upper barrier means 11; a sampling means 15A comprised of a shaft 15B and a surface sampling means 15C; a sampling means engaging means 13B which may optionally fit inside of the upper section 14A of the lower housing 14C; a separation means 17 separating the upper section 14A of lower housing 14C from a lower section 14B of the lower housing 14C; a signal means packaging means 18. In Figure 1, the top housing 10 and upper barrier 11 define a chamber 19 which optionally may contain a sealed or partially sealed container as shown in Figure 3.

Figure 2 illustrates a top housing 20, an upper barrier means 21 between the top housing 20 and the upper section 22 of a lower housing 27. The top housing 20 and upper barrier means 21 define a chamber 28 which optionally may contain a sealed or partially sealed container as shown in Figure 3. A sampling means 23. A separation means 24 separating the upper section 22 of lower housing 27 from a lower section 25 of the lower housing 27. A signal means packaging means 26.

Figure 3 illustrates alternative embodiments for providing means for packaging a fluid or dry substance in the chamber 28. Figure 3A illustrates a container 29 within chamber 28. Figure 3B illustrates a fluid 30 directly contained by the walls of the top housing 20 and upper barrier means 21. Figure 3C illustrates two containers 29A and 29B containing the same or different fluids. Figure 3D illustrates two containers 29C and 29A, 29C containing a dry substance and 29A a fluid. Figure 3E illustrates a fluid 30 directly contained by the walls of the top housing 20 and upper barrier means 21 and a container 29C containing a dry substance.

Figure 4 illustrates alternative embodiments for providing a sampling means to sample surfaces or fluids or

gases. Figure 4A illustrates a shaft 40A with a central channel 40B and channels 40C extending into a surface sampling means 41. Figure 4B illustrates a solid shaft 42 attached to a surface sampling means 43. Figure 4C illustrates a pipetting means 44 for sampling fluids. Figure 4D illustrates that a separate sampling means, such as a pipette 45 may be used.

Figure 5 illustrates alternative embodiments for providing a separation means. Figure 5A illustrates a semi-porous filter element 50. Figure 5B illustrates a one-way valving means. Figure 5C illustrates a puncturable membrane 52 and a puncturing device 53. Figure 5D illustrates a porous separation means 54. Figure 5E illustrates a temperature or solvent dissolvable membrane 55.

Figures 6A to 6H illustrate alternative embodiments for providing means for packaging a fluid or dry substance in a chamber 60 of the lower section 25 of lower housing 27. Figure 6A illustrates a container 61 containing a fluid or dry substance within chamber 60. Figure 6B illustrates a plastic or foil packet 62 containing a fluid or dry substance within chamber 60. Figure 6C illustrates a dry substance 63 directly contained within chamber 60. Figure 6D illustrates a fluid 64 directly contained within chamber 60. Figure 6E illustrates an ampule 65 within an ampule 66 or a packet 66 within a packet 66. The ampule or packet 65 may contain a fluid or dry substance. The ampule or packet 66 may contain a fluid or dry substance. The fluid or dry substance in ampule or packet 65 or 66 may be the same or different. Figure 6F illustrates an ampule 67 and an ampule 68 or a packet 67 and a packet 68. The ampule or packet 67 may contain a fluid or dry substance. The ampule or packet 68 may contain a fluid or dry substance. The fluid or dry substance in ampule or packet 67 or 68 may be the same or different. Figure 6G illustrates a fluid 69B directly contained in chamber 60 and a container 69A containing a fluid or dry substance.

Figure 6H illustrates an ampule or packet 70 containing a first substance 70A and a second substance 70B.

It will be readily apparent to one skilled in the art that various substitutions and modification may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The present invention, in one embodiment, describes a device and methods for the rapid and convenient testing of surfaces, solutions or gases for biological contamination by assaying for the presence of adenosine triphosphate ("ATP").

As described briefly above, the "sampling means washing means" may comprise for example, a reservoir containing a fluid in which the fluid may be selectively released after a sample has been obtained. The fluid may comprise the following: water, surfactants, salts, a neutralizing compound, such as sodium thiosulfate. More specifically, a suitable formulation is as follows: 50 mM Trizma base, 0.5 mM $MgCl_2$, 0.5% w/v Tween 80, 0.01 g/L Sodium Thiosulfate, pH to about 7.7 to 8.0 with, for example, acetic acid, a preservative, such as Rohm and Haas' Proclin 300 may be added to about 10-40 ppm, preferably 15 ppm. Further, an additional preservative, such as sodium Azide, may be added to 0.02% w/v.

Also as briefly described above a "signal generating means" such as a luciferase/luciferin composition may be used. Specifically, a suitable luciferase/luciferin composition comprises the following: Luciferase at a concentration of from about 50-100 µg/ml (the higher end of this range is preferable); Luciferin at a concentration of from about 0.1-4.0 mg/ml (the middle to high end of this range is preferable); EDTA at a concentration of from about 0.1 - 2.0 mM, (the middle of this range is preferable); DTT at a concentration of from about 2.0 - 4.0 mM (the middle of this range is preferable); BSA at about 5.0 - 50.0 mg/ml, (the middle of this range is preferred); Trizma at about 25 - 75 mM, (the middle of

this range is preferred); $MgCl_2$ at a concentration of about 25.0 - 100.0 mM (the middle of this range is preferred). Other buffer compositions may be suitably used. A preferred composition would be as follows: Luciferase 76 µg/ml, BSA 25 mg/ml, Luciferin 2 mg/ml, EDTA 1mM, DTT 3 mM, Trizma 50 mM, $MgCl_2$ 50 mM. The luciferase/luciferin composition is preferably lyophilized and stably packaged. Specifically, preparation of a luciferase/luciferin composition suitable for use in one embodiment of this invention may be done as follows: a 25 µl aliquot of the "luciferase/luciferin cocktail", (the luciferase/luciferin cocktail compositions are obtainable, for example, from Sigma chemical corporation or JBL or Boehringer Mannheim; by "cocktail" is meant the luciferin and luciferase and other elements that allow the system to produce a signal upon exposure to ATP), is deposited and frozen in a thin-walled glass onion-skin tube ("OST"). The aliquot is lyophilized to a moisture content of about less than 4%. The OST is then removed from the lyophilizer to a dry environment where the ends of the OST are melted in a high temperature flame, hermetically sealing the material inside the glass ampule.

Other specific applications of the invention are as follows:

(1) The testing of water samples to determine if they contain biological material. The procedure utilized to test for biological material in a water sample would be similar to the procedure used to test a surface, with the difference being that the sample tested is a liquid.

(2) The testing of samples for toxic material or compounds having bacteriocidal activity. Two suitable procedures are as follows: First, bacteria which produce bacterial luciferase, "bioluminescent bacteria" would be lyophilized as is known in the art. These bioluminescent bacteria would be placed into a stable packaging means in the device of the present invention. A sample would be added, as described elsewhere regarding the operation of

the device. Upon reconstitution these bioluminescent bacteria will "bioluminesce", thereby indicating their viability. Upon addition of a sample containing a toxic material of a particular threshold concentration the bacteria will cease exhibiting bioluminescence, indicating the presence of a toxic material. Bacterial reagents as are known in the art, for example the Microtox® reagent provided by Microbics Corporation of Carlsbad, California may be utilized. Ecotoxicology Monitoring, Section 3, Chapters 13-23, Mervyn Richardson Editor, (1993) is hereby incorporated by reference.

Alternatively, the luciferase gene may be cloned behind a promoter that is activated by various substances, for example, heavy metals. Upon the addition of a sample containing activating toxic material, for example, heavy metals, luciferase will be produced providing a detectable signal. Construction of a bacterial plasmid may be done as is known in the art and as set forth in Molecular Cloning, a Laboratory Manual Second Edition, Sambrook et al., incorporated herein by reference. Heavy metal inducible promoters and/or bacterial bioluminescent sensor strategies are disclosed in the following publications: Abstracts of the American Society for Microbiology, 93 general Meeting 16-20 May 1993, Atlanta Georgia; Abstract No. Q-95 Development of Bioluminescent Biosensors for Copper and Mercury, Gangolli et al.; Abstract No. Q-98 Biosensors Utilizing Promoterless Xenorhabdus luminescens lux for Detection of Mercury, Other Divalent Cations, and Selected Organics, Taylor et al.; Abstract No. Q-99 Transfer of an AlgD-lux Reporter Plasmid to Environmental Bacterial Strains and Demonstration of Bioluminescence in Relation to Factors Known to Influence Exopolysaccharide Synthesis, Rice et al.; Pollutant Detection by Induction of a Heat Shock Promoter-lux Gene Fusion in Escherichia coli K12, Van Dyk et al.; Stuart, "A 12-Base-Pair DNA Motif That is Repeated Several Times in Metallothionein Gene Promoters Confers Metal Regulation to a Heterologous

Gene," 81 *Proc. Natl. Acad. Sci. USA* 7318-7322, 1984, all of the above incorporated by reference herein.

(3) In another embodiment of the invention the testing of samples for specific procaryotic bacterial species (*e.g.*, Salmonella, *E. coli*, Listeria) or eucaryotic organisms such as yeast is performed. In this aspect of the invention, the signal generating means comprises a nutrient-indicator media, such as Colilert® nutrient-indicator media, available from IDEXX Laboratories of Westbrook, Maine. United States Patent 4,925,789 detailing the composition and use of nutrient-indicator media is incorporated herein by reference. A liquid sample is obtained with for example a pipette. The sample is mixed with the nutrient-indicator media. The whole device is incubated at a required temperature, dependent on the microorganism which one wishes to detect, for a specified time. For example, 35 degrees Centigrade for *E. coli*. If specific species of target bacteria are present a signal will be generated. More specifically, *E. coli* contain the enzyme β-glucorindase, while coliform bacteria contain the enzyme β-galactosidase. In the presence of the nutrient-indicator 4-methylumbelliferyl-β-D-glucuronide (MUG), *E. coli* will metabolize this compound, releasing an indicator portion, which when cleaved fluoresces when excited by an ultraviolet light source. Alternatively, coliform, in the presence of the nutrient-indicator o-nitrophenyl-β-D-galactoside (ONPG) will metabolize this compound, releasing an indicator portion, which when cleaved colors a sample yellow.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All such patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. One skilled in the art will readily appreciate that the present invention is well adapted to carry

out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods, procedures, treatments, and devices described herein are presently representative of preferred embodiments, are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

Claims

What is claimed is:

1. A device comprising:

(a) a sampling means;

(b) a sampling means washing means wherein said sampling means is contained within a housing means providing protection from pre-testing contamination for said sampling means;

(c) a signal generating means, wherein said signal generating means is stably packaged;

(d) a separation means for separating said signal generating means from a sample washed from said sampling means by said sample means washing means until the mixing of sample and signal generating means is desired; and

(e) a sample and signal generating means mixing means, wherein said elements (c)-(e) are contained in a lower housing, said lower housing divided into an upper and lower section by element (d).

2. The device of claim 1, wherein said sampling means is a swab.

3. The device of claim 1, wherein said sampling means is a pipette.

4. The device of claim 1, wherein said sampling means is a capillary tube.

5. The device of claim 1, wherein said sampling means washing means is a fluid which may be released from said upper housing containing said fluid.

6. The device of claim 1, wherein said signal generating means is a luciferase/luciferin composition.

7. The device of claim 6, wherein said luciferase/luciferin composition comprises: Luciferase at a concentration of from about 50-100 μg/ml; Luciferin at a concentration of from about 0.1-4.0 mg/ml and a suitable buffer composition.

8. The device of claim 6, wherein said luciferase/luciferin composition comprises: Luciferase at a concentration of from about 50-100 μg/ml; Luciferin at a concentration of from about 0.1-4.0 mg/ml; EDTA at a concentration of from about 0.1 - 2.0 mM; DTT at a concentration of from about 2.0 - 4.0 mM; BSA at about 5.0 - 50.0 mg/ml; Trizma at about 25 - 75 mM; $MgCl_2$ at a concentration of about 25.0 - 100.0 mM.

9. The device of claim 6, wherein said signal luciferase/luciferin composition is stably packaged in a moisture proof container.

10. The device of claim 6, wherein said signal luciferase/luciferin composition is stably packaged in a glass ampule.

11. The device of claim 1, wherein said signal generating means is a nutrient-indicator composition.

12. The device of claim 10, wherein said nutrient-indicator composition is Colilert©.

13. The device of claim 10, wherein said nutrient-indicator composition is stably packaged in a glass ampule.

14. The device of claim 1, wherein said signal generating means are bioluminescent bacteria.

15. The device of claim 13, wherein said bioluminescent bacteria produce a signal upon exposure to a toxic material.

16. The device of claim 14, wherein said signal produced upon exposure to a toxic material is bioluminescence.

17. The device of claim 14, wherein said signal produced upon exposure to a toxic material is the cessation of or decrease of bioluminescence.

18. The device of claim 1, wherein said separation means is a semi-porous filter.

19. The device of claim 1, wherein said separation means is a porous filter.

20. The device of claim 1, wherein said separation means is a puncturable membrane.

21. The device of claim 1, wherein said separation means is a temperature dissolvable membrane.

22. The device of claim 1, wherein said separation means is a chemical dissolvable membrane.

23. The device of claim 1, wherein said sample and signal generating means mixing means, comprises said lower section of said lower housing, wherein said lower section of said lower housing is deformable and upon compression allows for the creation of a pressure differential which upon release of the housing drives a sample washed from said sampling means by said sample means washing means into said lower section of said lower housing.

24. A device comprising:

(a) a receptacle for containing a fluid;

(b) said receptacle further comprising a breakable shaft which upon breakage exposes an orifice through which the fluid may flow;

(c) said receptacle adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(d) a swab means comprising a hollow shaft and an absorbent tip wherein said hollow shaft is in communication with said orifice in said receptacle;

(e) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(f) a moisture proof sealed vessel containing a lyophilized luciferase/luciferin composition contained in said lower section of said lower housing.

25. A device comprising:

(a) a pipette means wherein said pipette means is adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(b) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(c) a moisture proof sealed vessel containing lyophilized bioluminescent bacteria contained in said lower section of said lower housing, wherein said lower section of said lower housing further comprises a bacterial regeneration fluid.

26. The device of claim 25 wherein said bioluminescent bacteria are Microtox® bioluminescent bacteria.

27. A device comprising:

(a) a receptacle for containing a fluid;

(b) said receptacle further comprising a breakable shaft which upon breakage exposes an orifice through which the fluid may flow;

(c) said receptacle adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(d) a swab means comprising a hollow shaft and an absorbent tip wherein said hollow shaft is in communication with said orifice in said receptacle;

(e) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(f) a moisture proof sealed vessel containing nutrient-indicator media contained in said lower section of said lower housing.

28. The device of claim 26 wherein said nutrient-indicator media is Colilert® nutrient indicator media.

29. A device comprising:

(a) a pipette means wherein said pipette means is adjoined to a slidably engageable fitting, said fitting having a an inner member and an outer member between which a lower housing may slidably engage;

(b) a lower housing comprising an upper and a lower section said upper and lower sections separated by a semi-porous filter; and

(c) a moisture proof sealed vessel containing nutrient-indicator media contained in said lower section of said lower housing.

30. The device of claim 28 wherein said nutrient-indicator media is Colilert® nutrient indicator media.

31. A method for testing for the presence of biological matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with a signal generating means allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

32. A method for testing for the presence of biological matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with a nutrient-indicator composition, allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

33. A method for testing for the presence of toxic matter comprising the steps of sampling a surface, solution or atmosphere with a sampling means, washing said sampling means to remove at least a portion of any biological matter from said sampling means to generate a test sample, mixing said test sample with bioluminescent bacteria, allowing for reading of said signal generated, wherein said method is accomplished with a single self-contained testing device.

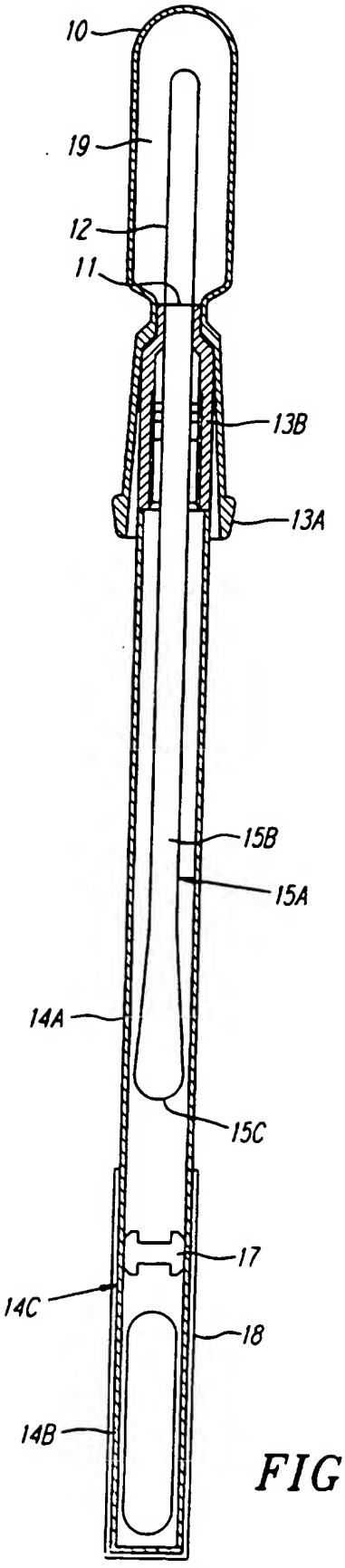

FIG. 1

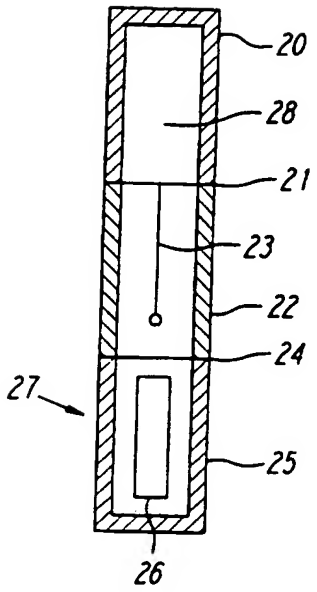

FIG. 2

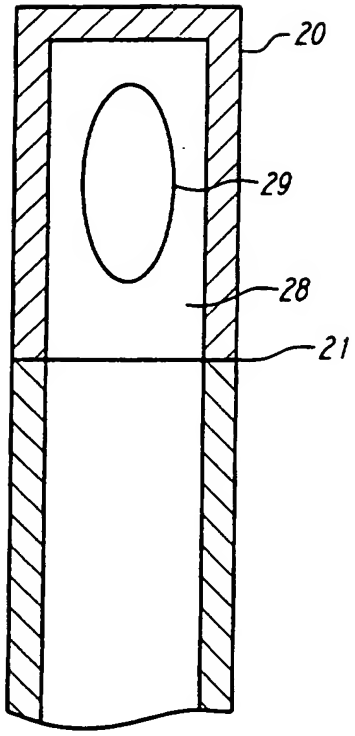

FIG. 3A

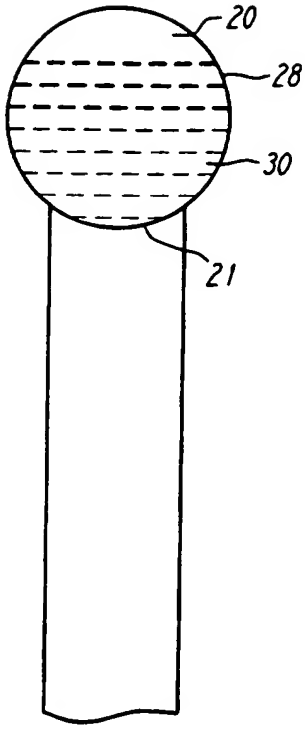

FIG. 3B

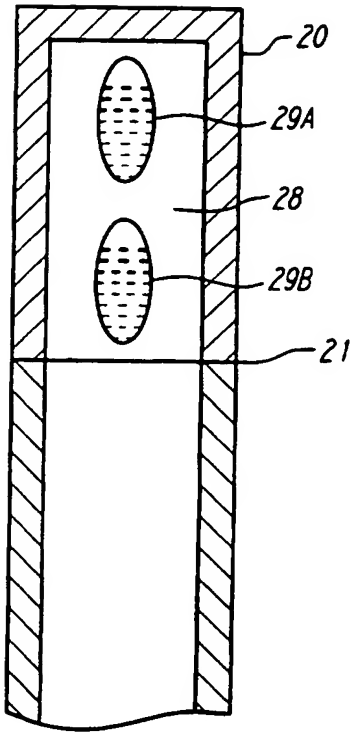

FIG. 3C

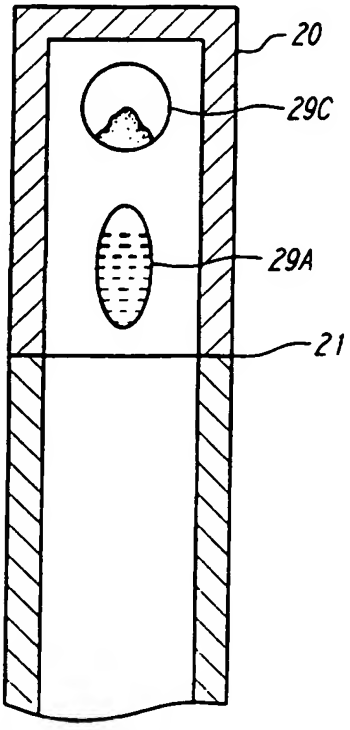

FIG. 3D

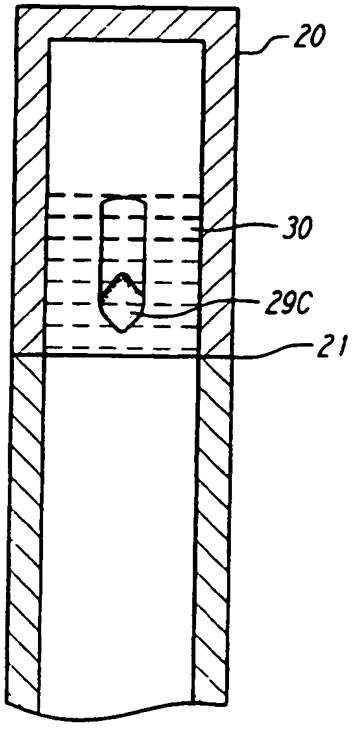

FIG. 3E

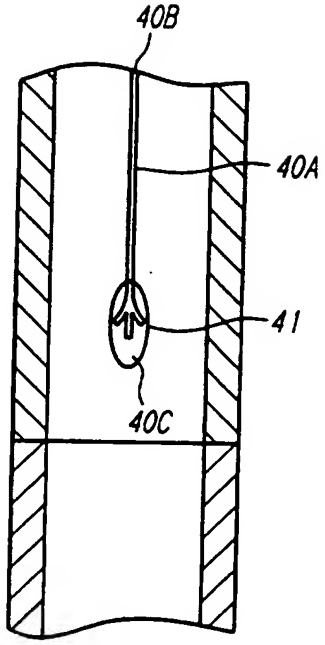

FIG. 4A

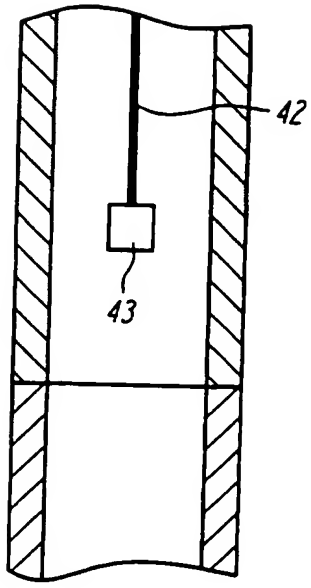

FIG. 4B

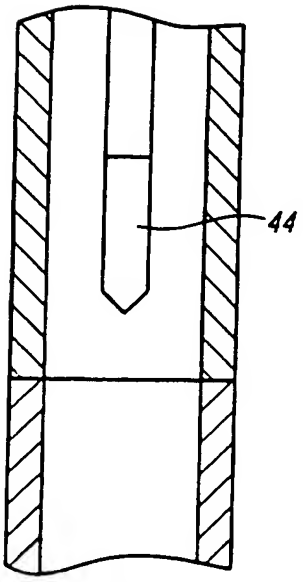

FIG. 4C

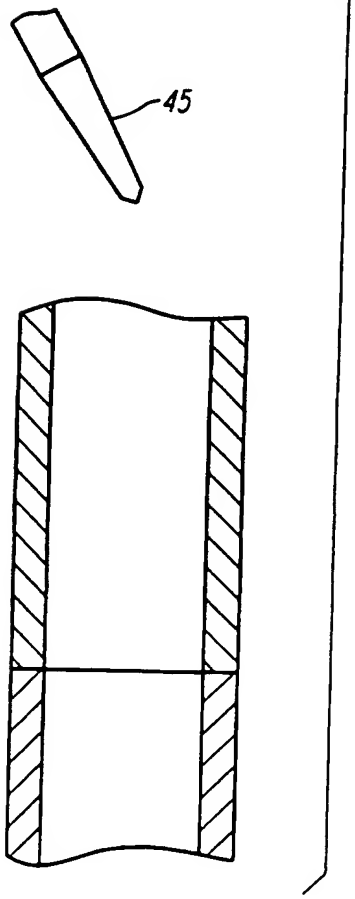

FIG. 4D

FIG. 5A

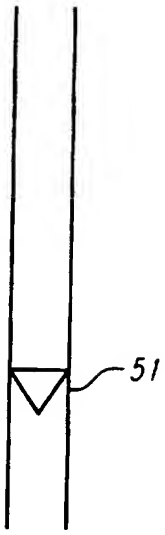

FIG. 5B

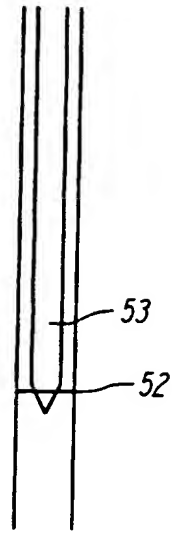

FIG. 5C

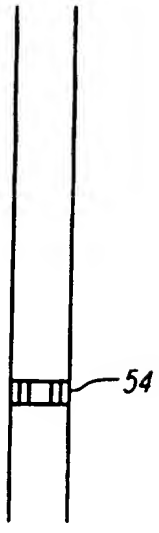

FIG. 5D

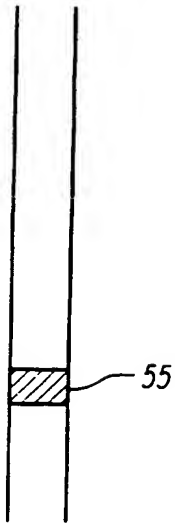

FIG. 5E

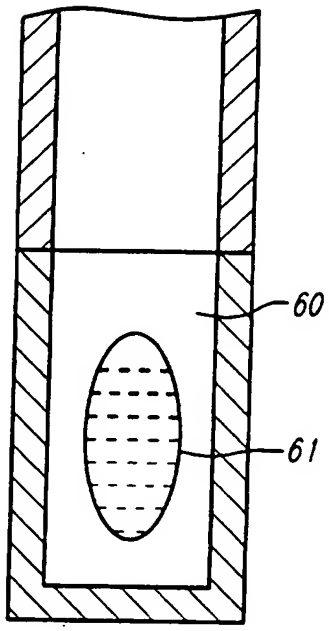

FIG. 6A

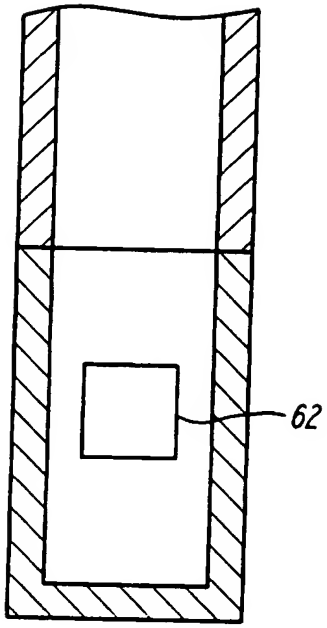

FIG. 6B

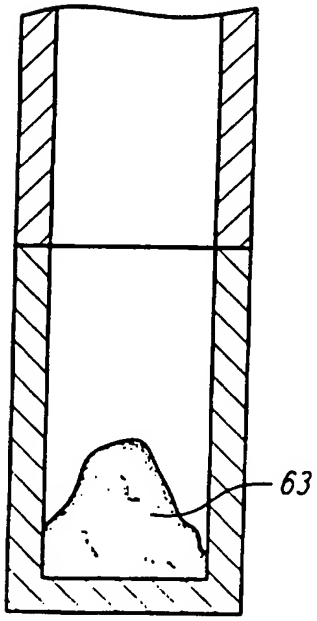

FIG. 6C

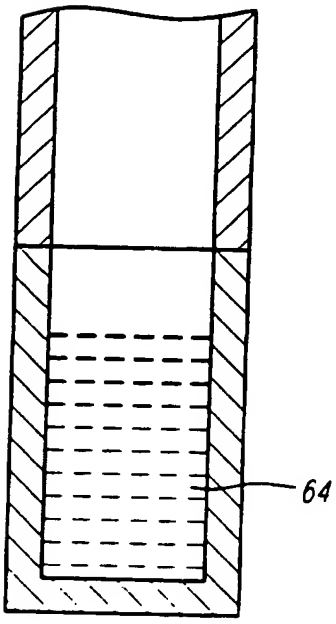

FIG. 6D

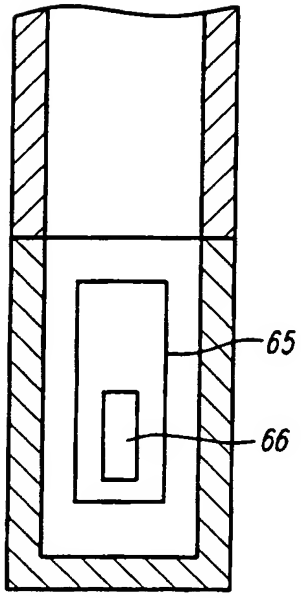

FIG. 6E

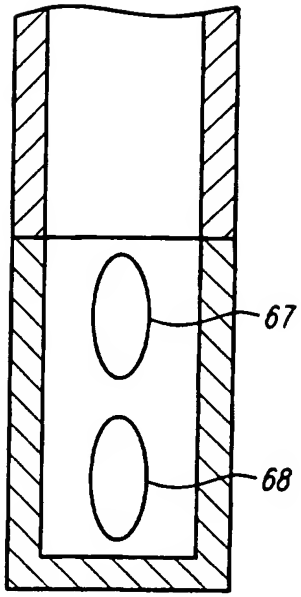

FIG. 6F

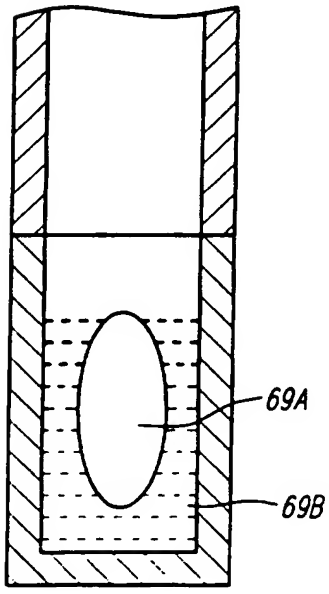

FIG. 6G

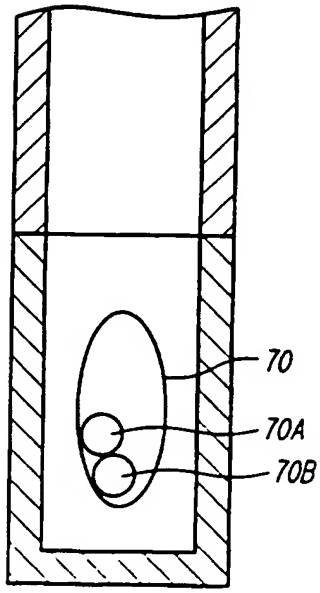

FIG. 6H

INTERNATIONAL SEARCH REPORT

Inte ɔnal Application No
PCT/US 95/13147

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/76 G01N1/02 B01L3/00 C12M1/30 A61B10/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L C12M G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 12421 (NASON) 24 June 1993	1,2,5, 18,19, 23,31
Y	see page 7, paragraph 1 - paragraph 2; figures 1,2	3,4, 6-17, 20-22, 24,27, 32,33
	see page 12, last paragraph - page 13, paragraph 1; figure 8	
	see page 13, paragraph 2; figures 10-12	
	see page 15, paragraph 1 - paragraph 3; figure 13	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 1996	27.03.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hocquet, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	see page 11, paragraph 3; figure 4E	4
Y	US,A,5 188 965 (DIFCO) 23 February 1993 see column 1, line 59 - column 2, line 15 see column 3, line 9 - line 61 see column 5, line 45 - line 66	6-10,24
Y	US,A,4 207 394 (ALDRIDGE) 10 June 1980 see column 5, line 6 - line 22 see abstract	11-13, 27,29,32
Y	GB,A,2 005 018 (BECKMAN INSTRUMENTS) 11 April 1979 see abstract; claims 1,2,16,17	14-17, 25,33
Y	PATENT ABSTRACTS OF JAPAN vol. 14 no. 306 (C-0735) ,3 July 1990 & JP,A,02 104273 (RICOH) 17 April 1990, see abstract	21
Y	US,A,5 047 044 (SMITH) 10 September 1991 see column 33, line 7 - line 19; figure 20	22
P,X	WO,A,95 25948 (CELCIS) 28 September 1995 see claims 1-5; figure 4	1,2,6-10
A	US,A,5 313 959 (MONTHONY) 24 May 1994 see column 4, line 12 - line 21 see column 4, line 22 - line 55 see column 5, line 40 - column 7, line 50; figure 9	1-33
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